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QTL controlling partial resistance to *Stagonospora nodorum* leaf blotch in winter wheat cultivar Alba

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Summary

Stagonospora nodorum blotch (SNB) is an important foliar and glume disease in cereals. Inheritance of SNB resistance in wheat appears quantitative. The development of partially resistant cultivars seems to be the only effective way to combat the pathogen. Partial resistance components like length of incubation period (INC), disease severity (DIS) and length of latent period (LAT) were evaluated on a population of doubled-haploids derived from a cross between the partially resistant cultivar Alba and the susceptible cultivar Begra. Experiments were conducted in controlled environments and the fifth leaf was examined. Molecular analyses were based on bulked segregant analyses (BSA) and screening with 240 microsatellites DNA markers. The QTL analysis revealed QTL on chromosome 6AL (designated as *QSnl.ihar-6A*) and putative QTL on chromosome 6D. The *QSnl.ihar-6A* accounted for 36% of the phenotypic variance for DIS and 14% for INC. The putative QTL accounted for 10% of the variability in INC and 8% of DIS components of SNB resistance.

Abbreviations: BSA: bulked segregant analysis; CAPS: cleaved amplified polymorphic sequences; CTAB: cetyltrimethylammonium bromide; DIS: disease severity; DH: doubled-haploid; INC: incubation period; LAT: latent period; PCR: polymerase chain reaction; QTL: quantitative trait loci; RFLP: restriction fragment length polymorphism; RGA: resistance gene analog; SNB: *Stagonospora nodorum* blotch; STS: sequence tagged site

Introduction

The fungus *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (anamorph: *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano) is a foliar and glume pathogen of wheat (*Triticum aestivum* L.) as well as of other cereals and grasses (Weber, 1922; Sprague, 1950; King et al., 1983; Scharen et al., 1985). The *Stagonospora nodorum* blotch (SNB) reduces yield and grain quality.

A high level of resistance to SNB in commercial cereals is not available (Shaner & Buechley, 1994). Alien germplasm may also not be a useful source of resistance (Ma & Hughes, 1993). Except in one reported common wheat line (Frecha, 1973; Kleijer et al., 1977), several *T. timopheevii*-derived durum wheat lines (Ma & Hughes, 1995), and a diploid wheat progenitor (*Aegilops tauschii*) (Murphy et al., 2000), inheritance of SNB resistance is quantitative and additive (Nelson, 1980; Ecker et al., 1989; Wilkinson et al., 1990; Wicki et al., 1999). In some wheat cultivars, seedling and adult plant responses to SNB are not correlated (Mullaney et al., 1983). SNB resistance tends to be higher in tall, late-maturing cultivars (Rosielle &

Brown, 1980; Scott et al., 1982). Response to SNB on spikes is mostly independent of response in leaves, and could be controlled by different genes (Fried & Meister, 1987; Bostwick et al., 1993). Since SNB resistance in common wheat is quantitative, several components of partial resistance including incubation period, latent period, infectious period, sporulation intensity, infection frequency, lesion size and number and percent leaf area with lesions help to discern genetic effects (Jeger et al., 1983; Lancashire & Jones, 1985; Griffiths & Jones, 1987; Cunfer et al., 1988; Loughman et al., 1996). Therefore, traditional breeding for SNB resistance in cases of large genotype × environment interaction, lack of effective resistance genes in different genetic backgrounds and different expressions of resistance depending on plant age make selection extremely difficult, if based only on phenotype analysis.

A more perspective approach called marker-assisted selection has emerged in recent years due to developments in molecular marker technology especially those based on the polymerase chain reaction (PCR; Mullis et al., 1986). Once the molecular markers closely linked to desirable traits are identified, marker-assisted selection can be performed in early generation segregating populations and at an early stage of plant development. Among different marker systems available, microsatellites (Litt & Luty, 1989) became the most useful for gene mapping in wheat, because they detect a large amount of DNA polymorphism (Plaschke et al., 1995; Bryan et al., 1997; Röder et al., 1998; Pestsova et al., 2000b; Prasad et al., 2000; Stachel et al., 2000). Molecular mapping experiments can be simplified by using bulked segregant analysis (BSA; Michelmore et al., 1991) and this approach is also useful for mapping quantitative resistance loci in wheat (William et al., 1997; Bai et al., 1999; Chantret et al., 2000). Single genes for resistance to *S. nodorum* were tagged with molecular markers in common wheat (Ellerbrook et al., 1999), durum wheat (Cao et al., 2001) and *Ae. tauschii* (Murphy et al., 1999). Quantitative resistance in wheat to *S. nodorum* using molecular markers and QTL analysis were recently explored by Czembor et al. (2003a, b) and in an earlier study in *T. spelta* (Messmer et al., 1997). The objective of our study was to detect QTLs associated with partial resistance components in the winter wheat cultivar Alba. For mapping, we created a doubled-haploid (DH) population from a cross between the partially resistant cultivar Alba and the susceptible cultivar Begra.

Materials and methods

Plant material and disease response phenotype

Winter wheat cultivars with partial SNB resistance (Alba) and susceptibility (Begra) (seed samples are available from the local gene bank, http://www.ihar.edu.pl/gene_bank/) were selected for crossing (Arseniuk et al., 1995). One hundred and thirty one doubled-haploid (DH) lines were developed from anther cultures of the F_1 generation. Partial resistance components, namely incubation period, latent period and disease severity were evaluated for parents and DH lines in controlled environments as described earlier (Czembor et al., 2003a). The experiment was designed as a one-way experiment with three randomized blocks (replicates). Due to the limited space in the controlled growth chamber, tests for each replicate were conducted separately. However, due to a growth chamber failure, only two measures for LAT were made. Statistical analyses were conducted using Statistica Software 5.0 (StatSoft Polska Sp. z o.o., ul. Kraszewskiego 36, 30–110 Kraków, Poland). The analyses of each individual for incubation period and disease severity were conducted on an average of 13 seedlings (sown in single pots arranged in one row) per repetition. Five leaves per replicate were used to assess the length of the latent period and scores were not averaged for statistical analysis. The DH lines with the most extreme and consistent reaction to the pathogen for each partial resistance component were chosen for BSA.

Molecular markers and QTL analysis

PCR and electrophoresis conditions (for agarose and polyacrylamide based gels), markers (228 microsatellites and 20 sets of primers targeting resistance gene analogs) and their polymorphism evaluation in BSA experiments followed the procedure described by Czembor et al. (2003a). To provide additional molecular markers in regions of chromosomes 6A and 6D putatively linked to QTLs after initial QTL analysis (LOD threshold was set on 1.5), we tested microsatellites WMC250 and WMC256 (Varshney et al., 2000), BARC21 BARC37, BARC107, BARC113, BARC175 and BARC204 (www.scabusa.org/pdfs/BARC_SSRs_011101.html). Additionally, we tried to develop STS (sequence tagged site) markers from RFLP (restriction fragment length polymorphism) probes specific to chromosomes 6A and 6D.

Sequence information for a particular RFLP was acquired by sequencing both ends of the probe (bcd1510, cmwg669, gbx3317, ksuD27, mwg813, R518, gwm-934) or from sequence information deposited in the Graingenes database (www.graingenes.org) (abc154 and BE439191). Monomorphic STS fragments were converted into cleaved amplified polymorphic sequence (CAPS) markers as described by Czembor et al. (2003a). These efforts resulted in two polymorphic markers: mwg934 (amplified in PCR by primers: 5'-GTG TCG TCC AGG TAG TAC TT-3' and 5'-CCG GCA CCA CCA ACT TTG AA-3') which revealed product length polymorphism, and ksuD27 (amplified in PCR by primers: 5'-TTT GCT GGT AAC GAA GCC CTA GC-3' and 5'-CTA TAT CCC AGC CCC TAG CTC TC-3') which showed polymorphism after digestion with the *Taq*I restriction enzyme.

Using bulked segregant analysis, we were able to identify markers potentially linked to QTLs that controlled resistance to *S. nodorum* leaf blotch. These single markers were quickly scanned using an interval mapping method (MapQTL 4.0, option "none" as the map file, Van Ooijen & Maliepaard, 1996) with LOD threshold 1.5, that enabled us to construct only linkage groups (JoinMap 2.0, Stam & Van Ooijen, 1995) containing markers putatively associated with QTL of resistance. Established linkage groups were used for QTL mapping using the interval mapping method with an LOD threshold 3.0.

Results

Disease response phenotype

Parental cultivars differed significantly ($p = 0.05$) only for DIS, but not for the other two resistance components (INC and LAT) (Figure 1). DH offspring lines differed significantly for each component studied. A relatively broad range of variation was found for each component, but parental cultivars were not extreme in their reaction to the pathogen especially regarding INC and LAT. Sixteen DH lines significantly ($p = 0.05$) transgressed the resistant parent 'Alba' in the length of incubation period. Regarding LAT, a reversed tendency was observed between parents (but this was not significant), since the resistant parent 'Alba' showed a shorter length of latent period (susceptibility) compared to 'Begra' (Figure 1). Seven DH lines significantly transgressed the range of LAT determined by the reaction of the parents. Mean values for repetitions (blocks) were very similar for DIS (4.7, 5.0 and 4.8 on 0–9 digit scale) and less consistent for INC (4.0, 2.4 and 3.6 days) and LAT (19.1 and 23.2 days). Correlation between average values for each component and scores for each repetition

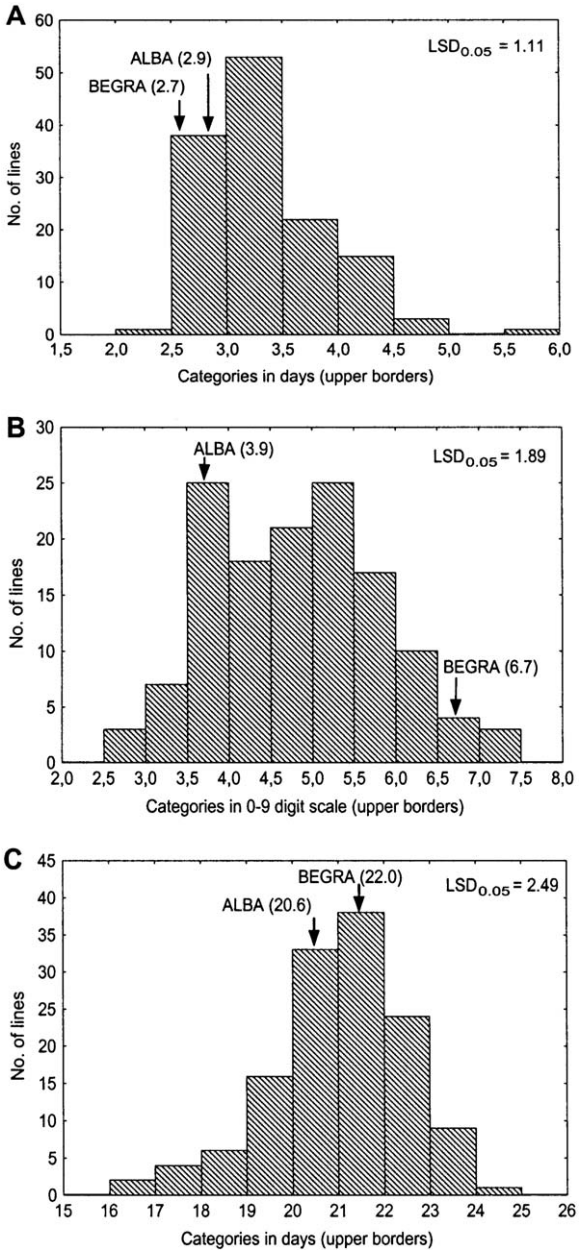

Figure 1. Distribution of 131 DH lines, from resistant parent 'Alba' × susceptible parent 'Begra' for resistance components (average values) to *Stagonospora nodorum*, namely incubation period (A) disease severity (B) and latent period (C).

varied from 0.63 to 0.8. We found a negative correlation between DIS and INC (-0.56, $p < 0.01$) or LAT (-0.38, $p < 0.01$), and a weak positive correlation between LAT and INC (0.21, $p < 0.05$).

Bulked segregant analysis and molecular markers

Using 20 sets of RGA primers, we did not detect any polymorphism between the parents. The 240 pairs of primers used in this study could potentially amplify 291 microsatellite loci as reported by Pestsova et al. (2000a) and Röder et al. (1998). We detected 255 microsatellite loci, of which, 114 were polymorphic between the parents (<50%). Only 16 microsatellite loci were polymorphic both between parents and between bulks within at least one of the three sets of bulks established for each resistance component. None of the 16 selected microsatellites could unequivocally distinguish the five resistant from the five susceptible DH lines used to establish alternative bulks. Furthermore, only three microsatellites (gdm98, gwm570 and gwm397) showed agreement between disease phenotype and molecular marker phenotype for at least eight of 10 DH lines. Marker gwm397, however, was not considered in further analysis, because it had a LOD value lower than 1.5 after initial QTL searching. According to the literature (Röder et al., 1998; Pestsova et al., 2000a) the remaining two markers, gwm570 and gdm98, were mapped on chromosomes 6A and 6D, respectively. These two microsatellites along with a number of additional polymorphic markers were used to further analyze chromosome 6A (gwm459, gwm617 and mwg934) and 6D (gdm108, BARC21, BARC175 and ksuD27). After analysis of segregation data based upon an expected 1:1 segregation ratio, the following markers were eliminated from the data set ($p < 0.05$): BARC21 ($\chi^2 = 36.91$), ksuD27 ($\chi^2 = 43.6$), gwm459 ($\chi^2 = 61.54$) and gwm617 ($\chi^2 = 37.49$). Two linkage groups corresponding to chromosomes 6A (gwm570 and mwg934) and 6D (gdm98, gdm108 and BARC175) were subjected further for QTL analysis.

QTL analysis

Based upon the LOD curves calculated for the partial genetic maps of 6A and 6D chromosomes, we found a significant QTL on chromosome 6A and a putative (LOD value below 3.0) QTL on 6D (Figure 2). According to the rules for designation of identified genes or QTLs in wheat (McIntosh et al., 1998), we named QTL on 6A chromosome as *QSnl.ihar-6A*. This QTL was significantly associated with INC (LOD = 4.08) and DIS (LOD = 7.26) (Figure 2A), and explained 14% and 36% of the phenotypic variances, respectively. The second QTL located on the distal part of chromosome 6DL was associated with lower significance levels, since LOD values 2.85 and 2.23 were below the critical LOD threshold 3.0 (Figure 2B). This putative QTL explained 10% and 8% of the phenotypic variance for INC and DIS, respectively. Both, *QSnl.ihar-6A* and the putative QTL on 6D chromosome were not associated with LAT (Figure 2).

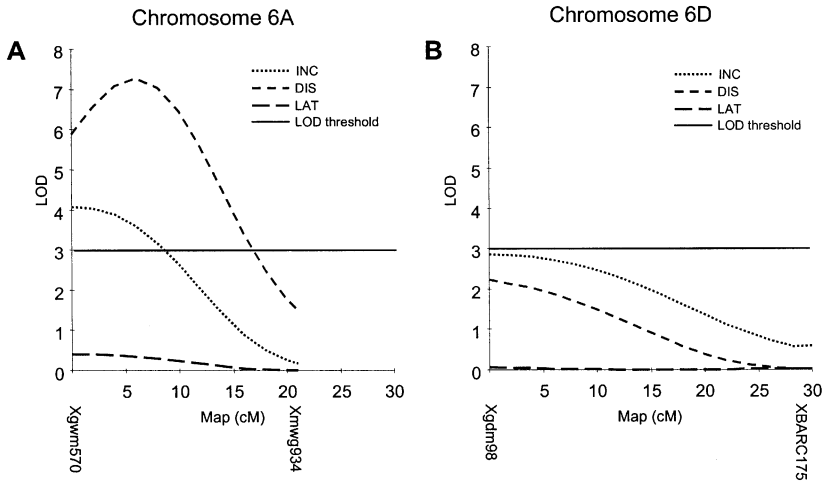

Figure 2. LOD curves based on simple interval mapping of QTLs for incubation period (INC), disease severity (DIS) and latent period (LAT) on linkage groups corresponding to wheat chromosomes 6A (A) and 6D (B).

Discussion

The quantitative resistance to SNB found in our study is in agreement with the general concept of partial resistance to the disease determined by several components (Jeger et al., 1983; Lancashire & Jones, 1985; Griffiths & Jones, 1987; Cunfer et al., 1988; Loughman et al., 1996). We found two QTLs associated with partial resistance components located on chromosomes 6A and possibly 6D. These chromosomes were already shown to be involved in disease resistance studies using monosomic lines (Kleijer et al., 1977; Waląg & Dzięgło, 1985, 1987; Ariau et al., 1988) and chromosomal substitution lines (Kleijer et al., 1980). Chromosomes 6A and 6D (Kleijer et al., 1980; Waląg & Dzięgło, 1987; Auriau et al., 1988) were found to be implicated in resistance as gene(s) located on these chromosomes could increase resistance reaction expressed by gene(s) on other chromosomes (Kleijer et al., 1977; Waląg & Dzięgło, 1985). Partial resistance of line 'L22' to *S. nodorum* studied by Auriau et al. (1988) was controlled by five chromosomes of which four were related to incubation period. Among them, chromosome 6D was specified. These results indicate that the present putative QTL detected on that chromosome could represent another allele or gene associated with a longer incubation period and lower disease severity. Several QTLs for disease resistance are already mapped to chromosome 6A. *QSnl.ihar-6A* is localized at the chromosomal region where QTL for quantitative expression of leaf tip necrosis (*QLtn.sfr-6A*) was also detected (Messmer et al., 2000). *QLtn.sfr-6A* is pleiotropic or closely linked with quantitative leaf rust resistance in wheat. A QTL corresponding to *MlRE* for resistance to powdery mildew was mapped distally to *QSnl.ihar-6A* (Chantret et al., 2000).

QSnl.ihar-6A was strongly affected by DIS, to a lesser extent by INC and not at all by LAT. Certainly, there was a large amount of variation in these resistance components that remained unexplained. This could be due to QTLs that remain undetected or because no markers were identified in regions associated with those QTLs. Enormous difficulties were encountered in establishing linkage groups due to a lack of polymorphism and frequent deviation of markers from the expected 1:1 Mendelian ratio. The low number of molecular markers available for QTL analysis was due to a narrow genetic background of our mapping population that negatively influenced the level of DNA polymorphism detected. Both parents, 'Begra' (= Grana × Bezostaya 1) and 'Alba' (= Maris Hantsmann × L 83270; where L 83270 = [(Weigue × Dańkowska Biała) × Luna] × Grana) have a common ancestor in their pedigrees, cultivar Grana.

Quantitative resistance to *S. nodorum* using molecular markers was explored in a population of recombinant inbred lines derived from a wide cross between *T. spelta* cultivar Oberkulmer and *T. aestivum* cultivar Forno (Messmer et al., 1997). Seven QTLs were detected in *T. spelta* for resistance to leaf infection each explaining 5–13% of the phenotypic variance, but only two were consistent over 3 years. In our study, *QSnl.ihar-6A* explained a much higher proportion of phenotypic variance of lower disease severity (up to 36%) and a similar proportion for longer incubation period (up to 14%). The effect of the *QSnl.ihar-6A* on resistance to SNB is similar to that represented by the recently detected four QTLs on chromosomes 2B, 3B, 5B and 5D in wheat cultivar Liwilla at fifth-leaf stage (Czembor et al., 2003a). In that study, the percentage of phenotypic variance explained by a single QTL ranged from 14–21% for the INC, from 16–37% for DIS, and from 13–28% for LAT (Czembor et al., 2003a).

Preliminary results of the field evaluation of two QTLs described in our work indicated that none of them was associated with leaf resistance in adult plants (Czembor et al., 2003b). However, head resistance was consistently associated with the QTL mapped on chromosome 6D. The percentage of phenotypic variance explained by this QTL was 27% and 25%, respectively, in field trials during 2 years (Czembor et al., 2003b). Further work aimed at detection of more QTLs associated with field resistance in adult plant leaves and heads is required to understand resistance mechanisms in different plant organs and at various growth stages.

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References

Ariau, P., F. Rapilly & Y. Cauderon, 1988. Monosomic analysis of resistance to *Septoria nodorum* Berk. in soft wheat (*Triticum aestivum* L. em Thell (Vill) M.K.). Agronomie 8: 71–77.

Arseniuk, E., H.J. Czembor, W. Sowa, H. Krysiak & J. Zimny, 1995. Genotypic reaction of triticale, wheat and rye to inoculation with *Stagonospora* (=*Septoria*) *nodorum* under field conditions and *S. nodorum* and *S. tritici* under controlled environment. Biuletyn IHAR 195/196: 209–246.

Bai, G., F.L. Kolb, G. Shaner & L.L. Domier, 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89: 343–348.

Bostwick, E.D., H.W. Ohm & G. Shaner, 1993. Inheritance of *Septoria nodorum* blotch resistance in wheat. Crop Sci 33: 439–443.

Bryan, G.J., A.J. Collins, P. Stephenson, A. Orry, J.B. Smith & M.D. Gale, 1997. Isolation and characterization of microsatellites from hexaploid bread wheat. Theor Appl Genet 94: 557–563.

Cao, W., G.R. Hughes, H. Ma & Z. Dong, 2001. Identification of molecular markers for resistance to *Septoria nodorum* blotch in durum wheat. Theor Appl Genet 102: 551–554.

Chantret, N., P. Sourdille, M.S. Röder, M. Tavaud, M. Bernard & G. Doussinault, 2000. Location and mapping of the powdery mildew resistance gene *MlRE* and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. Theor Appl Genet 100: 1217–1224.

Cunfer, B.M., D.E. Stookbury & J.W. Johnson, 1988. Components of partial resistance to *Leptosphaeria nodorum* among seven soft red winter wheats. Euphytica 37: 129–140.

Czembor, P.C., E. Arseniuk, A. Czaplicki, Q.J. Song, P.B. Cregan & P.P. Ueng, 2003a. QTL mapping of partial resistance in winter wheat to *Stagonospora nodorum* blotch. Genome 46: 546–554.

Czembor, P.C., E. Arseniuk & P.P. Ueng, 2003b. Field evaluation of quantitative trait loci (QTLs) associated with partial resistance to *Phaeosphaeria nodorum* in wheat seedlings. In: G.H.J. Kema, M. van Ginkel & M. Harrabi (Eds.), Proc 6th International Symposium on Septoria and Stagonospora Diseases of Cereals. Global insights into the Septoria and Stagonospora Diseases of Cereals, 8–12 December 2003, Tunis, Tunisia, pp. 119–122.

Ecker, R., A. Dinoor & A. Cahaner, 1989. The inheritance of resistance to *Septoria* glume blotch. I. Common bread wheat, *Triticum aestivum*. Plant Breed 102: 113–121.

Ellerbrook, C.M., V. Korzun & A.J. Worland, 1999. Using precise genetic stocks to investigate the control of *Stagonospora nodorum* resistance in wheat. In: M. van Ginkel, A. McNab & J. Krupinsky (Eds.), Proc 5th International Septoria Workshop. Septoria and Stagonospora Diseases of Cereals: A Compilation of Global Research, CIMMYT, Mexico D.F., pp. 150–153.

Frecha, J.H., 1973. The inheritance of resistance to *Septoria nodorum* in wheat. Boletin Genetico del Instituto de Fitotecnia, Castelar 8: 29–30.

Fried, P.M. & E. Meister, 1987. Inheritance of leaf and head resistance of winter wheat to *Septoria nodorum* in diallel cross. Phytopathology 77: 1371–1375.

Griffiths, H.M. & D.G. Jones, 1987. Components of partial resistance as criteria for identifying resistance. Ann Appl Biol 110: 603–610.

Jeger, M.J., D.G. Jones & E. Griffiths, 1983. Components of partial resistance of wheat seedlings to *Septoria nodorum*. Euphytica 32: 575–584.

King, J.E., R.J. Cook & S.C. Melville, 1983. A review of *Septoria* diseases of wheat and barley. Ann Appl Biol 103: 345–373.

Kleijer, G., A. Bronnimann & A. Fossati, 1977. Chromosomal location of a dominant gene for resistance at the seedling stage to *Septoria nodorum* Berk. in the wheat variety Atlass 66. Z Pflanzenzüchtg 78: 170–173.

Kleijer, G., A. Fossati & F.X. Paccaud, 1980. Genetic analysis of tolerance to *Septoria nodorum* Berk. in wheat using chromosome substitution lines. Z Pflanzenzüchtg 85: 287–293.

Lancashire, P.D. & D.G. Jones, 1985. Components of partial resistance to *Septoria nodorum* in winter wheat. Ann Appl Biol 106: 541–553.

Litt, M. & J.A. Luty, 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44: 397–401.

Loughman, R., R.E. Wilson & G.J. Thomas, 1996. Components of resistance to *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* in spring wheats. Euphytica 89: 377–385.

Ma, H. & G.R. Hughes, 1993. Resistance to *Septoria nodorum* blotch in several *Triticum* species. Euphytica 70: 151–157.

Ma, H. & G.R. Hughes, 1995. Genetic control and chromosomal location of *Triticum timopheevii* derived resistance to septoria nodorum blotch in durum wheat. Genome 38: 332–338.

McIntosh, R.A., G.E. Hart, K.M. Devos, M.D. Gale, W.J. Rogers, J. Dubcovsky & C.F. Morris, 1998. Catalogue of Gene Symbols for Wheat (http://wheat.pw.usda.gov/index.shtml).

Messmer, M., M. Keller, M. Winzeler, G. Schachermayr, C. Feuillet, F. Gallego, H. Winzeler & B. Keller, 1997. Identification of quantitative trait loci (QTL) for *Septoria nodorum* resistance in a wheat by spelt population. Programme and Abstracts of International *Triticae* Mapping Initiative (ITMI) Public Workshop, June 25–27, 1997. National de la Recherche Agronomique (INRA), Centre de Clermont-Theix, Station d'amelioration des plantes, Clermont-Ferrad, France, p. S12.

Messmer, M., R. Seyfarth, M. Keller, G. Schachermayr, M. Winzeler, S. Zanetti, C. Feuillet & B. Keller, 2000. Genetic analysis of durable leaf rust resistance in winter wheat. Theor Appl Genet 100: 419–431.

Michelmore, R.W., I. Paran & R.V. Kesseli, 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88: 9828–9832.

Mullaney, E.J., A.L. Scharen & M.D. Bryan, 1983. Resistance to *Septoria nodorum* in a durum wheat cultivar as determined by stage of host development. Can J Bot 61: 2248–2250.

Mullis, K., S. Faloona, S. Scharf, R. Saiki, G. Horn & H. Erlich, 1986. Specific enzymatic amplification of DNA *in vitro*: The polymerase chain reaction. Cold Spring Harb Symp Quant Biol 51: 263–273.

Murphy, N.E.A., R. Loughman, R. Wilson, E.S. Lagudah, R. Appels & M.G.K. Jones, 1999. Identification of a molecular marker linked to septoria nodorum blotch resistance in *Triticum tauschii* using F_2 bulked segregant. In: M. van Ginkel, A. McNab & J. Krupinsky (Eds.), Proc 5th International Septoria Workshop. Septoria and Stagonospora Diseases of Cereals: A

Compilation of Global Research, CIMMYT, Mexico D.F., pp. 71–73.

Murphy, N.E.A., R. Loughman, R. Wilson, E.S. Lagudah, R. Appels & M.G.K. Jones, 2000. Resistance to septoria nodorum blotch in the *Aegilops tauschii* accesion RL5271 is controlled by a single gene. Euphytica 113: 227–233.

Nelson, L.R., 1980. Inheritance of resistance to *Septoria nodorum* in wheat. Crop Sci 20: 447–449.

Pestsova, E., M.W. Ganal & M.S. Röder, 2000a. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43: 689–697.

Pestsova, E., V. Korzun, N.P. Goncharov, K. Hammer, M.W. Ganal & M.S. Röder, 2000b. Microsatellite analysis of *Aegilops tauschii* germplasm. Theor Appl Genet 101: 100–106.

Plaschke, J., M.W. Ganal & M.S. Röder, 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91: 1001–1007.

Prasad, M., R.K. Varshney, J.K. Roy, H.S. Balyan & P.K. Gupta, 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet 100: 584–592.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy & M.W. Ganal, 1998. A microsatellite map of wheat. Genetics 149: 2007–2023.

Rosielle, A.A. & A.G.P. Brown, 1980. Selection for resistance to *Septoria nodorum* in wheat. Euphytica 29: 337–346.

Scharen, A.L., Z. Eyal, M.D. Huffman & J.M. Prescott, 1985. The distribution and frequence of virulence genes in geographically separated populations of *Leptosphaeria nodorum*. Phytopathology 75: 1463–1468.

Scott, P.R., P.W. Benedikz & C.J. Cox, 1982. A genetic study of relationship between height, time of ear emergence and resistance to *Septoria nodorum* in wheat. Plant Pathol 31: 45–60.

Shaner, G. & G. Buechley, 1994. Field evaluation of resistance to septoria leaf blotch. Hodowla Roślin Aklimatyzacja i Nasiennictwo 38: 173–176.

Sprague, R., 1950. Diseases of Cereals and Grasses in North America. Ronald Press, New York, 583 pp.

Stachel, M., T. Lelley, H. Grausgruber & J. Vollmann, 2000. Application of microsatellites in wheat (*Triticum aestivum* L.) for studying genetic differentiation caused by selection for adaptation and use. Theor Appl Genet 100: 242–248.

Stam, P. & J.W. Van Ooijen, 1995. JoinMap^{TM} version 2.0: Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen.

Van Ooijen, J.W. & C. Maliepaard, 1996. MapQTL^{TM} version 3.0: Software for calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen.

Varshney, R.K., A. Kumar, H.S. Balyan, J.K. Roy, M. Prasad & P.K. Gupta, 2000. Characterization of microsatellites and development of chromosome specific STMS markers in bread wheat. Plant Mol Biol Rep 18: 5–16.

Walag, H. & A. Dzięgło, 1985. Monosomic location of genes for resistance to *Septoria nodorum* (Berk.) in the spring wheat (*Triticum aestivum* L.) variety Sappo. Hodowla Roślin Aklimatyzacja i Nasiennictwo 29: 11–19.

Walag, H. & A. Dzięgło, 1987. Location of resistance genes to *Septoria nodorum* (Berk.) of spring wheat of the Weibull 15440 variety (*Triticum aestivum* L.). Hodowla Roślin Aklimatyzacja i Nasiennictwo 31: 1–6.

Weber, G.F., 1922. II. *Septoria* diseases of wheat. Phytopathology 12: 537–585 with plates XXIII–XXXVI.

Wicki, W., M. Winzeler, J.E. Schmidt & P. Stamp, 1999. Inheritance of resistance to leaf and glume blotch caused by *Septoria nodorum* Berk, in winter wheat. Theor Appl Genet 99: 1265–1272.

Wilkinson, C.A., J.R. Murphy & R.C. Rufty, 1990. Diallel analysis of components of partial resistance to *Septoria nodorum* in wheat. Plant Dis 74: 47–50.

William, H.M., D. Hoisington, R.P. Singh & D. Gonzales-de-Leon, 1997. Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 34: 479–501.